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14. ABSTRACT

Based on our published data we propose that specific patterns of genomic copy number alterations (CNAs) occur in prostate cancer in African American (AA) men which are associated with different levels of disease aggressiveness. Second, specific patterns of gene expression are associated with disease aggressiveness in AA prostate cancer and these reflect, in part, the specific CNAs at the relevant gene loci. Finally, we propose that 4p16.3, which is lost in 30% of AA prostate cancers, contains one or more tumor suppressor genes that impact prostate cancer initiation and progression in AA men. This proposal will test these hypotheses by carrying out high throughput copy number and expression microarray analysis of DNAs and RNAs from cancer and benign tissues from African American men with prostate followed by an in depth analysis of the 4p16.3 region.

15. SUBJECT TERMS
None provided.

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INTRODUCTION

African American (AA) men have both a higher incidence and significantly higher mortality rates from prostate cancer (PCa) than Caucasian (CAU) men[1]. To what extent racial differences observed in PCa incidence and mortality are due to socioeconomic or biological factors remains controversial. Several groups have found that AA patients exhibited greater tumor volumes in comparison to similarly staged CAU patients [2, 3]. A major factor that has inhibited understanding the unique biology of PCa in AA men is the lack of clinical and pathological resources focused specifically on this problem. The vast majority of PCa molecular genetic and biological studies do not take differences in race into account when analyzing the results. This reflects the general under-representation of AA men in such studies, which substantially weakens the statistical power of any sub-group analysis. This is exacerbated by the generally lower percentage of AA patients in most tertiary referral centers where most such studies are performed. While some of the difference in mortality due to PCa can be attributed to socioeconomic factors, a number of studies have shown that there is a still a higher mortality rate from prostate cancer in AA men even after adjustment for socioeconomic factors [4]. Thus, as concluded by Freedland and Isaacs[4], that in addition to socioeconomic and cultural factors, biological differences account for some of the disparity in incidence and mortality for prostate cancer in AA men in comparison to CAU men. **The central problem addressed in this project is to understand the biological basis for the more aggressive clinical behavior of PCa in AA men and to develop predictive tools to help manage PCa in AA men.**

We have analyzed 20 PCas from AA men with high density single nucleotide polymorphism arrays [5] to detect genomic copy number alterations (CNAs). Comparison of our primary tumors with tumors from CAU patients from a previously published cohort with similar pathological characteristics showed higher frequency of loss of at numerous loci, all of which had higher frequencies in metastatic lesions in this previously published cohort. **This difference may in part explain the more aggressive clinical behavior of prostate cancer in AA men and indicates that AA men will need specific prognostic tools based on the biology of their PCa.** Furthermore, when we performed cluster analysis of CNAs with both AA and CAU patients, almost all of the AA patients fell into two clusters, one associated with less aggressive organ confined disease and a second associated with more aggressive, invasive disease. **This is an exciting finding indicating that analysis of CNAs and patterns of CNAs may have prognostic value in AA men with PCa.** Finally, we indentified a novel region on chromosome 4p16.3 that is lost in 30% of AA PCas which has not been previously shown to be lost in PCa. This region has previously shown to be lost in breast, colon and bladder cancer and harbors several potential tumor suppressor genes.

We hypothesize that specific patterns of CNAs occur in AA PCa which are associated with different levels of disease aggressiveness. Second, we hypothesize that specific patterns of gene expression are associated with disease aggressiveness in AA PCa and these reflect in part the specific CNAs at the relevant gene loci. Finally, we hypothesize that 4p16.3, which is lost in 30% of AA PCa, contains one or more tumor suppressor genes that impact PCa initiation and progression in AA men. This proposal will test theses hypotheses by carrying out the tasks outlined below.

BODY

Task 1: High resolution analysis of genomic alterations in African American prostate cancers.

Subtask 1: Sixty pairs of samples will be obtained from the Baylor Prostate Cancer Tissue Bank. Samples will be from African American (AA) men undergoing radical prostatectomy for treatment of prostate cancer and were collected with informed consent. Prostate cancer (PCa) samples will have 80% tumor and will have a matched benign tissue available from the same patient. DNA and RNA will be extracted by standard methodologies. Assess DNA and RNA integrity by standard techniques. **(Months 1-2)**

Progress: We hav**e** extracted DNA and RNA from 69 pairs of prostate cancer and benign tisues. Quality was assessed and is high as shown in Figure 1. We have also identified additional tissues for extraction which is underway.

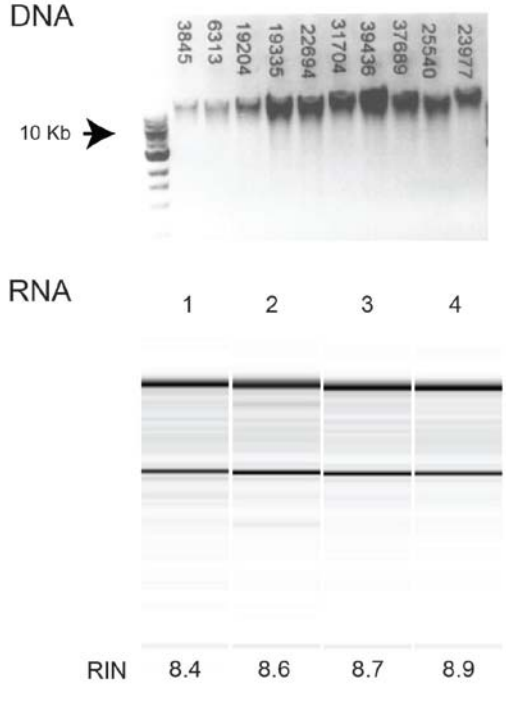

Figure 1. DNA and RNA from African American prostate cancer and prostate tissues.

Top: agarose gel of DNA showing undegraded high molecular weight DNA' The 10 Kb marker is shown on the left. Numbers are sample IDs.

Bottom: Agilent Bioanalyzer analysis of RNA. RNA integrity numbers (RIN) are shown at the bottom of each lane. A RIN of > 7 is required for optimal analysis by expression microarrays.

Subtask 2: Copy number analysis of DNAs from PCa and benign tissues from AA prostate cancers will be carried out as we have described previously except using Affymetrix 6.0 SNP arrays. **(Months 3-18)**

Progress: The Affymetrix 6.0 Arrays are in process and will be completed by month 18

Subtask 3: Continuous quality control of data **(Months 3-18)**

Progress: We are carrying out continuous quality assurance.

Subtask 4: Data analysis for copy number alterations in PCas from AA. Hierarchical clustering (complete linkage method) of copy gain/loss profiles of prostate tumors from: AA PCa (new dataset); all Baylor AA PCa (new and old dataset); all AA PCa (our datasets and published AA datasets). We will also compare our AA cases (new and old) and the published CAU datasets. **(Months 18-36)**

Progress: To be performed when CNA data completed

Subtask 5: We will determine the extent to which any CNA or pattern of CNAs is associated with PSA recurrence using both the new dataset and the combined dataset (new plus prior study) using Cox proportional hazard regression modeling of biochemical recurrence to develop multivariate survival models with specific CNA cluster groups, specific CNAs and/or groups of CNAs. **(Months 18-36)**

Progress: To be performed when CNA data completed

Task 2: Whole genome expression array analysis in African American prostate cancers.

Subtask 1. Expression array analysis of prostate cancers from AA men using RNAs extracted from PCa tissues containing 80% or more tumor from AA men in Task 1 above and matching benign tissues. We will use human whole genome arrays from Agilent for expression microarray analysis as described previously. Each of these arrays contains 60-mer oligos that can detect 41,000 transcripts corresponding to the known human transcriptome. **(3-18 months)**

Progress: RNAs extracted and expression arrays are in progress (See Fig 1 above).

Subtask 2. Continuous quality control of data **(3-18 months)**

Progress: We are carrying out continuous quality assurance.

Subtask 3. Initial data analysis using unsupervised cluster analysis of the AA expression dataset. Compare our data to existing publically available datasets available on the Web for both AA and CAU men. **(18-24 months)**

To be carried out after RNA expression analysis completed

Subtask 4: Correlate expression and CNA analysis **(18-36 months)**

To be carried out after RNA expression analysis completed

Subtask 5: Carry out pathway analysis of expression data **(18-36 months)**

To be carried out after RNA expression analysis completed

Subtask 6: Validation of key gene expression changes in PCa identified during data analysis using quantitative RT-PCR **(18-36 months)**

To be carried out after RNA expression analysis completed

Task 3: Identification of potential tumor suppressor gene(s) on 4p16.3 in AA PCa.

Subtask 1: Identify minimal deleted region on 4p16.3 by analysis of CNA data from Task 1. Identify any homozygous deletions. **(Months 18-21).**

Progress: To be performed when CNA data completed

Subtask 2: Further define minimal deleted region by analysis of gene expression data from Task 2 **(Months 18-21).**

Progress: To be performed when CNA data completed

Subtask 3: Exome capture of minimal deleted region defined in Sub-tasks 1 and 2 above and perform next generation sequencing to identify mutations **(Months 21-26)**

Progress: To be performed when CNA data completed

Subtask 4: In vitro functional studies of potential tumor suppressor genes identified based on homozygous deletion, mutation or decreased expression with relevant known or predicted biological functions. **(26-36 months)**

Progress: To be performed when CNA data completed

KEY RESEARCH ACCOMPLISHMENTS

- We have identified and extracted high quality DNAs and RNAs from matched cancer and benign tissues from 69 radical prostatectomies.
- Copy number and expression array analysis are in progress.
- Data analysis and mining, validation and detailed analysis of 4p16.3 will proceed after the large scale genomics is completed.

REPORTABLE OUTCOMES

- None to date; large scale data acquisition in progress

CONCLUSION

We have acquired and extracted the required samples to provide a comprehensive picture of the genomic landscape in African American prostate cancer. Such analysis is proceeding and will provide a wealth of data over the next 12 months.

REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ: Cancer statistics, 2009, CA Cancer J Clin 2009, 59:225-249
2. Sanchez-Ortiz RF, Troncoso P, Babaian RJ, Lloreta J, Johnston DA, Pettaway CA: African-American men with nonpalpable prostate cancer exhibit greater tumor volume than matched white men, Cancer 2006, 107:75-82
3. Moul JW, Connelly RR, Mooneyhan RM, Zhang W, Sesterhenn IA, Mostofi FK, McLeod DG: Racial differences in tumor volume and prostate specific antigen among radical prostatectomy patients, J Urol 1999, 162:394-397
4. Freedland SJ, Isaacs WB: Explaining racial differences in prostate cancer in the United States: sociology or biology?, Prostate 2005, 62:243-252
5. Castro P, Creighton CJ, Ozen M, Berel D, Mims MP, Ittmann M: Genomic profiling of prostate cancers from African American men, Neoplasia 2009, 11:305-312